THE ZEBRAFISH AS AN ANIMAL MODEL FOR DEVELOPMENT AND DISEASE RESEARCH

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P.T.

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National Institute of Diabetes and Digestive and Kidney Diseases
National Cancer Institute
National Center for Research Resources
National Eye Institute
National Heart, Lung, and Blood Institute
National Human Genome Research Institute
National Institute on Aging
National Institute of Alcohol Abuse and Alcoholism
National Institute of Allergy and Infectious Diseases
National Institute of Arthritis and Musculoskeletal and Skin Diseases
National Institute on Deafness and Other Communication Disorders
National Institute of Dental Research
National Institute on Drug Abuse
National Institute of Environmental Health Sciences
National Institute of General Medical Sciences
National Institute of Mental Health
National Institute of Neurological Disorders and Stroke

PURPOSE

The purpose of this Program Announcement (PA) is to solicit applications as part of a National Institutes of Health (NIH) initiative to increase our support of the zebrafish as an animal model for research. This PA is intended to continue stimulation of a trans-NIH initiative that was started with RFA: DK-98-006, entitled "Genomic Resources for the Zebrafish," NIH Guide for Grants and Contracts, Vol. 26, No. 39, December 5, 1997.

HEALTHY PEOPLE 2000

The Public Health Service (PHS) is committed to achieving the health promotion and disease prevention objectives of "Healthy People 2000," a PHS-led national activity for getting priority areas. Potential applicants may obtain a copy of "Healthy People 2000" (Full Report: Stock No. 017-001-00474-0 or Summary Report: Stock No. 017-001-00473-1) through the Superintendent of Documents, Government Printing Office, Washington, DC 20402-9325 (telephone: 202-512-1800).

ELIGIBILITY REQUIREMENTS

Applications may be submitted by domestic and foreign for-profit and non-profit organizations, public and private, such as universities, colleges, hospitals, laboratories, units of State and local governments, and eligible agencies of the Federal government. Racial/ethnic minority individuals, women, and persons with disabilities are encouraged to apply as Principal Investigators.

MECHANISM OF SUPPORT

The mechanism of support for this PA will be the NIH investigator-initiated research project grant (R01) award. Applications for R01s from new investigators are particularly encouraged. The total project period for an application submitted in response to this PA may not exceed five years.

Responsibility for the planning, direction, and execution of the proposed project will be solely that of the applicant. Applications will compete with all investigator-initiated applications and will be reviewed according to the customary peer review procedures. Awards will be administered under PHS grants policy as stated in the Public Health Service Grants Policy Statement.

This PA is the result of a trans-NIH initiative with participation of the Institutes and Centers listed above, working though the Cross-NIH Zebrafish Coordinating Committee, under the co-chairmanship of the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) and the National Institute of Child Health and Human Development (NICHD). The principal awards will be made through the Institute or Center whose mission is most closely related to the proposed work. Through the Cross-NIH Zebrafish Coordinating Committee, each Institute will share with the other participating Institutes, research supported as a result of this PA . All investigators funded under this initiative will be expected to work together cooperatively so that the information learned and the models developed will be of maximum usefulness to the community.

An applicant planning to submit a grant application requesting $500,000 or more in direct costs for any year is required to contact, in writing or by telephone, Institute or Center program staff when the application development process begins. Furthermore, the applicant must obtain agreement from Institute/Center staff that the Institute or Center will accept the application for consideration for award. The applicant Principal Investigator must identify, in a cover letter sent with the application, the program staff member and Institute or Center that has agreed to accept assignment of the application. An application received without indication of prior staff concurrence and identification of that contact will be returned to the applicant without review.

RESEARCH OBJECTIVES

Background

Vertebrate development has been characterized extensively using the methods of classical embryology, molecular biology and biochemistry. However, mutational analysis in vertebrates, has lagged behind such investigations in invertebrates. As experimentation in Drosophila melanogaster and Caenorhabditis elegans has established, mutational studies are a powerful tool to determine the events that result in patterning and morphogenesis of the embryo. When combined with genetic combinatorial analyses, mutational analyses can identify specific genes that act during embryonic development, provide insight into how they function, and clarify the pathways in which they participate. Studies that compare results from these invertebrate systems with those obtained in vertebrates have established that there is remarkable evolutionary conservation in the genetic programs that determine embryo formation, including such early patterning events as formation of the embryonic axes, but also including later events such as development of eye, heart, and other organs. Although invertebrate systems are extremely powerful and numerous aspects of development are conserved, many aspects of patterning and morphogenesis of the vertebrate embryo are distinct and cannot be studied in invertebrates. The vertebrate embryo has many features not present in other models, including the substantially different organization and greater complexity of the nervous system and the fact that some vertebrate organs have no clear cognates in the simpler invertebrates. Thus, understanding human development will require application of experimental approaches to the formation of the vertebrate embryo. Some assessment of mutations that affect development has been possible in the mouse, but the mouse embryo is inaccessible in utero throughout much of its development. Consequently, mutational studies in this species have been limited largely to defects in post-natal maturation. While reverse genetics (e.g., knock-outs) have been useful in the mouse model, the substantial costs of maintaining large mouse colonies has limited the applicability of forward genetic approaches, which will have a profound impact on our understanding of development.

As a vertebrate, the zebrafish, Danio rerio, is more closely related to humans than are yeast, worms or flies. It has a number of valuable features as a model organism for study of vertebrate development. Many features of zebrafish development have been characterized, including early embryonic patterning, early development of the nervous system, and aspects of cell fate and lineage determination. The embryos are transparent and accessible throughout development. In live embryos, the same specific cell or even cellular processes can in many cases be identified from individual to individual, affording a high level of precision in characterizing the effect of developmental or genetic perturbation. Techniques for ablation and transplantation of individual cells have been used to explore questions about induction and cell fate, and continue to be refined. There are also a growing number of molecular markers to facilitate developmental studies. Because of their relatively short reproductive cycle, the large number of progeny that can be produced, and the relatively small space needed to maintain large numbers of offspring, the zebrafish is an efficient vertebrate model system for genetic analysis. It is possible to generate haploid progeny, which are viable to the point where many recessive embryonic phenotypes can be identified, and also homozygous diploid progeny that carry only maternal (or paternal) genetic information. A genetic map of approximately 2-3 centimorgan resolution is available, and mutations can be readily placed on the map. Positional cloning of genes identified by mutation has recently been accomplished. Finally, there are several promising methods for transformation and insertional mutagenesis which are now being developed.

Two large-scale screens have been performed to date and the transparent embryos screened for defects in overall embryonic pattern, morphogenesis or organ formation. As reported in the December 1996 issue of Development, these screens have identified a substantial number of mutations that affect the formation of organ systems, including defects in the nervous system, skeletal muscle, craniofacial region, kidney and endocrine organs, cardiovascular and gastrointestinal systems, and the sensory cells of lateral line systems which are important to auditory and vestibular function. For most of these mutations, the gene defect has not yet been identified. It is likely that many of these mutations affect genes relevant to human development and disease processes such as cancer and neurodegenerative diseases. The zebrafish offers the opportunity of using classical genetics to define gene functions.

Scope

The objective of this PA is to promote the zebrafish as an animal model for the study of development and disease. The goals of this PA are to encourage new and innovative research and approaches using the zebrafish to identify the genes and elucidate the molecular and genetic mechanisms responsible for normal and defective development and disease.

Each of the participating Institutes and Centers has interests in using the zebrafish as a model system to better understand particular processes, organs, or diseases. In addition, some may be interested in supporting development of methods, either general techniques or techniques that may particularly apply to their areas of interest. Each Institute with their general statement of interest is listed below in alphabetical order. Some Institutes have included examples of research topics that are appropriate for this PA; however, they are not to be considered as exclusive or limiting.

Institute or Center Statement of Interest

NCI. Generation and study of zebrafish models to identify and place genes in functional pathways that affect growth and development; in particular, genes/pathways which, when altered, result in uncontrolled or cancerous growth.

NCRR. Research projects to broaden the utility of the zebrafish model. Projects should be applicable to a broad range of biomedical research and to more than one categorical NIH Institute or specific disease.

NEI. Fundamental mechanisms underlying all aspects of eye development, function and disease, including retinal development and optic axon guidance.

NHGRI. Proposals for the development of high throughput, widely applicable technologies or methodologies to examine gene function on a genomic scale. This could include initial development of high throughput or large-scale methods for examining gene expression, development of tools for comprehensive mutational analysis, or genome-scale identification of regulatory regions.

NHLBI. Cellular and molecular functions of the mutant genes in development as models for human cardiovascular, blood and pulmonary disorders, and circadian mechanisms underlying sleep/wake cycles. Genetic basis of disorders of cardiovascular development and function; developmental aspects of endothelial dysfunction as the basis for systemic and pulmonary vascular disorders; developmental defects in hematopoiesis and relationship to disorders of the hematopoietic system; genetic basis of vasculogenesis; effect of mutations on subsequent organ development leading to such disorders as lung hypoplasia and bronchopulmonary dysplasia; and the genetic basis of circadian rhythm.

NIA. Basic research on the genetic and molecular basis of aging and longevity. Generation and analysis of late-age onset or long-lived mutants that can be used to identify, clone, and characterize genes involved in normal and pathological aging. Cellular and molecular function of genes expressed in the aging nervous system as well as in other organ systems, such as the cardiovascular, immune, and musculoskeletal systems. Such genes include, but are not limited to, those involved in neurodegenerative disorders, neuroplasticity, cell death, damage and repair of DNA and proteins, and oxidative metabolism, and maintenance of differentiated cell function.

NIAAA. Mechanistic studies of ethanol-induced teratogenesis, behavioral impairments, and organ damage.

NIAID. Mechanisms underlying immune system development and function. Hematopoiesis, including the development of T cells, B cells, antigen presenting cells, and cells of the innate immune system, such as natural killer cells. Regulation of key immune cell molecules during development, such as regulation of genes encoding antigen receptors, co-receptors, cytokine receptors, and cytokines. Regulation of genes involved in mature immune cell function following antigen encounter, including regulation and role of cytokines, receptor expression, and any other determinants of humoral and cellular immunity following antigen administration.

NIAMS. Generation and analysis of mutant zebrafish that have the potential to illuminate the development and function of the vertebrate musculoskeletal system and skin. The musculoskeletal system includes muscle, bone, articulated joints, cartilage, tendon and ligament. Priority will be given to the establishment of collaborations between investigators with expertise in the zebrafish and investigators with expertise in the musculoskeletal systems and skin of mammals and humans.

NICHD. Identification, cloning, and characterization of the genes important in normal development as well as those mutant genes which cause developmental defects. Elucidation of the cellular, biochemical, molecular and genetic mechanisms underlying normal and defective development. This includes, but is not limited to the study of general mechanisms of pattern formation and cell lineage, cell specification, differentiation, migration and fate in early development of many organs/systems.

NIDA. Identification of mechanisms underlying tolerance, sensitization, and addiction to drugs of abuse such as nicotine, amphetamine, cocaine, opiates, barbiturates, and hallucinogens. Identification of genetic suppressors and enhancers of the teratological effects of drugs of abuse on behavior and the nervous system. Processes involved in the development of brain regions mediating the hedonic properties of drugs of abuse.

NIDCD. Identification and cloning of genes involved in the normal and disordered development of hearing, balance, smell and taste sensory systems. Elucidation of the cellular, molecular, and biochemical mechanisms governing the proliferative, plastic and regenerative capacities of these sensory cells and tissues.

NIDDK. Research on diabetes, other endocrine and metabolic diseases, disorders of hematopoeisis, and for diseases of the digestive system, liver, kidney, and urinary tract. Studies aiming to clarify the cellular and molecular events which dictate organ formation in all these systems are considered of relevance. These studies could include but need not be limited to studies to develop cell lines from any of the organs of interest, studies to characterize normal or abnormal function of organs of interest, methods to screen and identify additional mutations in these systems, studies to define the molecular mechanisms which dictate cell-specific gene expression in relevant cell types.

NIDR. All aspects of normal and abnormal craniofacial development, including genetics, complex origins of craniofacial disorders, cell lineages and differentiation, cell signaling and gene regulation, embryonic patterning, imaging, biomimetics, and new technologies for high-throughput genetic and protein screens.

NIEHS. Studies to examine the mechanism whereby environmental factors/agents alter development. Characterization of the interactions among genetics, environmental agents and time during development that lead to structural or functional abnormalities. Development of a mechanistically based model for testing environmental agents for developmental toxicity.

NIGMS. Basic biomedical research that addresses fundamental biological mechanisms such as those that underlie gene regulation, chromosome organization and mechanics, cell growth and differentiation, pattern formation, sex determination, morphogenesis, cell cycle control, behavior, the genetics of complex traits, and the application of mathematical models to complex biological systems.

NIMH. Investigations which examine molecular, cellular, and biochemical bases of genetic mutations affecting neurogenesis and mental behavior. These studies include, but are not limited to, screening for such mutants, identification, isolation, and mapping of the defective genes, and their functional analyses.

NINDS. Research on the development, normal function, and diseases of the nervous system. This research might include the use of mutants to understand the mechanisms controlling the following processes: neurogenesis, nervous system patterning, cell lineage, cell migration, programmed cell death, axon pathfinding and regeneration, myelination, neural crest cell development, and motor and sensory function. Also of interest would be research into the functional neuroanatomy of developing and adult nervous systems, the use of optical imaging techniques to visualize neural activity and development of methods for analyzing and perturbing gene function in single cells.

The areas of interest listed above are not in any order of priority. They are only examples of areas of research to consider. Applications with areas of interest to more than one Institute or Center will be assigned to multiple Institutes or Centers for funding consideration. Applicants are encouraged to propose other areas that are related to the objectives and scope of this PA.

APPLICATION PROCEDURES

Applications are to be submitted on the grant application form PHS 398 (rev. 5/95) and will be accepted on the standard application deadlines as indicated in the application kit. Requests for continued funding of already funded projects (Type 2) will be considered under this program announcement. Application kits are available at most institutional offices of sponsored research and may be obtained from the Division of Extramural Outreach and Information Resources, National Institutes of Health, 6701 Rockledge Drive, MSC 7910, Bethesda, MD 20892-7910, telephone 301/435-0714, email: Grantsinfo@nih.gov.

For identification and processing purposes, the PA number and title must be typed in item 2 of the application face page and the "YES" box must be marked.

Submit a signed, typewritten original of the application, including the Checklist, plus five signed photocopies, in one package to:

CENTER FOR SCIENTIFIC REVIEW
NATIONAL INSTITUTES OF HEALTH

6701 ROCKLEDGE DRIVE, ROOM 1040 - MSC 7710
BETHESDA, MD 20892-7710
BETHESDA, MD 20817 (for express/courier service)

REVIEW CONSIDERATIONS

Applications will be assigned on the basis of established PHS referral guidelines. Upon receipt, applications will be reviewed for completeness by Center for Scientific Review (CSR). Incomplete applications will be returned to the applicant without further consideration. Applications will be evaluated for scientific and technical merit by an appropriate peer review group convened in accordance with the standard NIH peer review procedures. As part of the initial merit review, all applications will receive a written critique and undergo a process in which only those applications deemed to have the highest scientific merit, generally the top half of applications under review, will be discussed, assigned a priority score, and receive a second level review by the appropriate national advisory council or board.

Review Criteria

The goals of NIH-supported research are to advance our understanding of biological systems, improve the control of disease, and enhance health. In the written review, comments on the following aspects of the application will be made in order to judge the likelihood that the proposed research will have a substantial impact on the pursuit of these goals. Each of these criteria will be addressed and considered in the assignment of the overall score.

o Significance: scientific, technical or medical significance and originality of the proposed research will be evaluated.

o Approach: Are the conceptual framework, design, methods, and analyses appropriate and adequate to accomplish the aims of the project? Does the applicant acknowledge potential problem areas and consider alternative tactics? Is the scientific and technical merit of the research proposed sufficient to advance the objectives of the PA?

o Innovation: Does the project employ novel concepts, approaches or method? Are the aims original and innovative? Does the project challenge existing paradigms or develop new methodologies or technologies?

o Investigator: Are the Principal Investigator and staff appropriately trained and well suited to carry out this work? Is the work proposed appropriate to the experience level of the principal investigator and other researchers (if any)?

o Environment: Does the scientific environment in which the work will be done contribute to the probability of success? Do the proposed experiments take advantage of unique features of the scientific environment or employ useful collaborative arrangements? Is there evidence of institutional support?

o Budget and duration: Are the proposed budget and duration appropriate in relation to the proposed research?

Availability of special opportunities for furthering research programs through the use of unusual talent resources, populations, or environmental conditions in other countries that are not readily available in the United States or that provide augmentation of existing U.S. resources will be considered in the review.

The initial review group also will examine the provisions for the protection of human and animal subjects, and the safety of the research environment.

AWARD CRITERIA

Factors that will be used to make award decisions are as follows:

o Quality of the proposed project as determined by peer review;

o Cost effectiveness of the proposed strategy;

o Promise of the proposed program to accomplish the goals of this PA and address the needs of the participating ICs as regards their interest in the zebrafish as a model organism;

o Availability of funds.

INQUIRIES

Written and telephone inquiries concerning this PA are encouraged. The opportunity to clarify any issues or questions from potential applicants is welcome.

Direct inquiries regarding programmatic issues to:

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AUTHORITY AND REGULATIONS

This program is described in the Catalog of Federal Domestic Assistance No.93.865 - Research for Mothers and Children, National Institute of Child Health and Human Development. Awards are made under authorization of the Public Health Service Act, Title IV, Part A (Public Law 78-410, as amended by Public Law 99-158, 42 USC 241 and 285) and administered under PHS grants policies and Federal Regulations 42 CFR 52 and 45 CFR Part 74. This program is not subject to the intergovernmental review requirements of Executive Order 12372 or Health Systems Agency review.

The PHS strongly encourages all grant and contract recipients to provide a smoke-free workplace and promote the non-use of all tobacco products. In addition, Public Law 103-227, the Pro-Children Act of 1994, prohibits smoking in certain facilities (or in some cases, any portion of a facility) in which regular or routine education, library, day care, health care or early childhood development services are provided to children. This is consistent with the PHS mission to protect and advance the physical and mental health of the American people.

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